Name ____ Period ________

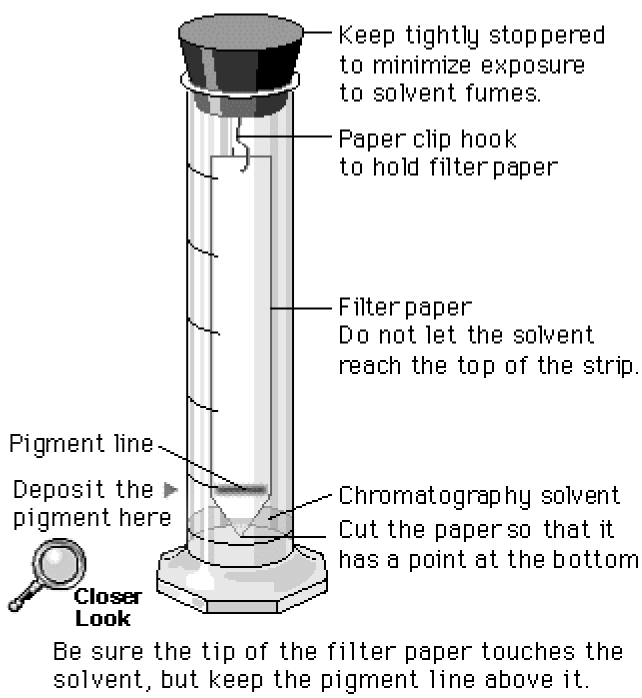
Light & Plant Pigments Lab

Pre-Lab Information & Analysis

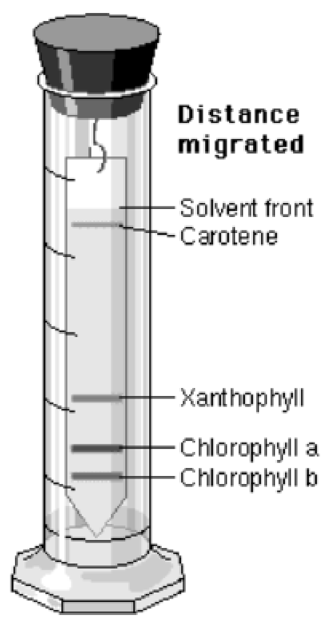
Paper chromatography is a technique used to separate a mixture into its component molecules. The molecules migrate, or move up the paper, at different rates because of **differences in solubility**, **molecular mass** (larger molecules move slower), and **hydrogen bonding with the paper** (many hydrogen bonds cause to adhere to paper more and thus move slower). Sometimes what appears to be a single color may in fact be a material composed of many different pigments —and such is the case with chloroplast pigments. Usually leaves are green but we will see that many green leaves actually have different colors of pigments.

In paper chromatography the pigments are dissolved in a solvent that carries them up the paper. To separate the pigments of the chloroplasts, you must use an organic solvent. In the lab activity, you will separate plant pigments using an organic solvent such as a mixture of ether and acetone since pigments are not water soluble, and therefore would not move up the paper with water. Be sure to keep the bottle tightly closed except when you are using it because ***the solvent is very volatile and produces fumes***

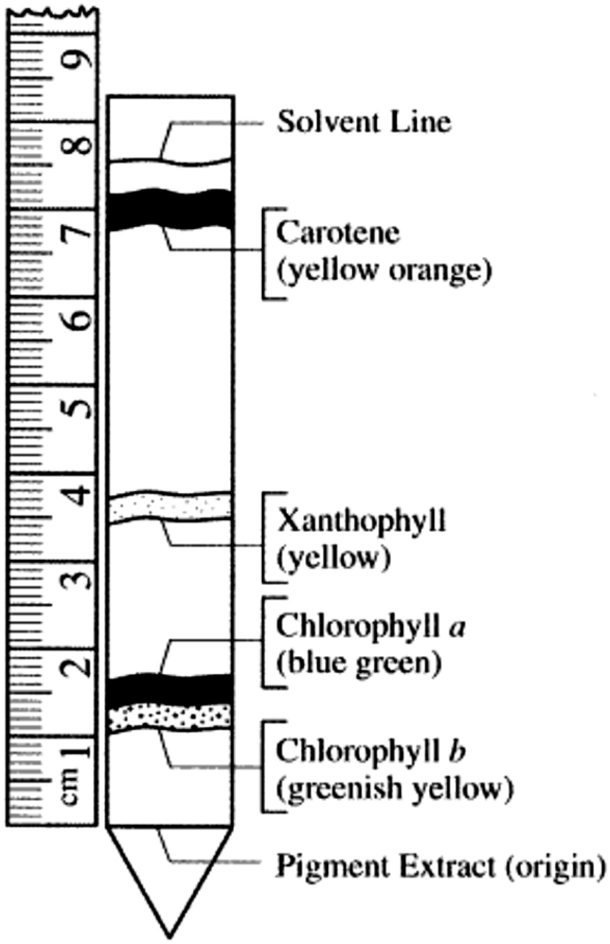
Beginning of Experiment



End of Experiment



Answer the following prompts below on separate paper for submission on lab day.



1. Biological molecules can be separated by using chromatographic techniques. The diagram above shows the separation of several spinach leaf pigments by paper chromatography. Using the diagram above

   (a) **Explain** how paper chromatography can be used to separate pigments based on their chemical and physical properties.

   (b) **Discuss** the role of pigments both in capturing light energy and in converting it to the chemical energy of ATP and NADPH.

   (c) Use the ruler shown above to **determine** the R_f value of xanthophyll. **Show** your calculations.

   R_f value = distance between pigment origin to migrated pigment band ÷ distance between pigment origin to solvent front. Note: Use the vertical center of the pigment bands to determine their numerical value.

Procedures

Step 1: Place a leaf across the paper and use a coin to rub the leaf pigments onto the paper. Let dry and repeat 2 more times, drying between each time.
Step 2: Set the chromatogram in the chromatography solvent. Make sure that the level of the solvent is below the origin on your chromatogram – **you do not want to submerge the pigment line in the solvent**.
Step 2: When the solvent "front" is within 2- 3 cm from the top of the paper, remove the chromatogram. Use a pencil to **quickly** mark the location of the solvent front. Allow the chromatogram to air dry, then outline each pigment band you observe (they will fade over time).
Step 3: Identify your pigments & Calculate their Rf values.

Identification of Pigments by Color
Anthocyanins – red/brownish, pink or purple
Chlorophyll a - blue-green/dark green
Chlorophyll b - yellow-green/light green
Xanthophyll – yellow
Carotene – yellow-orange

Conduct a Chi-Square analysis. The **Frequency observed** is how many of the 3 Rf values (yours & 2 classmates') were within the expected interval.

PIGMENTS	Rf Value ***Expected*** Interval	Observed Rf Values (Record 3 values including your own and 2 other classmates')			Frequency ***Observed*** out of 3 samples	Frequency ***Expected*** out of 3 samples
Chlorophyll b	0.10-0.26					**3**
Chlorophyll a	0.15-0.38					**3**
Anthocyanin	0.02-0.09					**3**
Xanthophyll	0.35-0.55					**3**
Carotene	0.80-0.99					**3**

Only include the pigment categories that were visible for all 3 samples. Compare to the .05 critical value at the appropriate degrees of freedom (# pigments -1).

Analysis Questions – REQUIRED LAB GRADE ASSIGNMENT

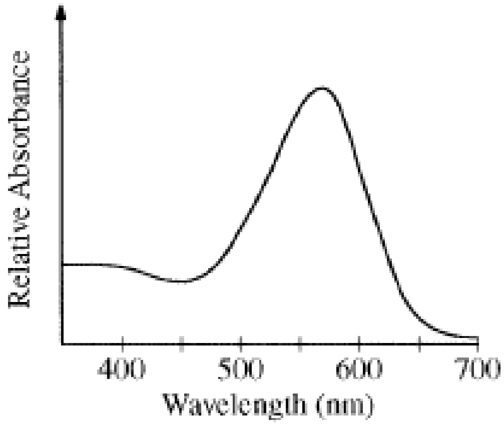
1. **Identify** what the null hypothesis is & **explain** whether it should be rejected or accepted, using your chi square results to justify your decision.

2. What are the **scientific implications** of your data? In other words, do your data support what the "standard data" are? If not, what might be the **reason(s) for the deviation**?

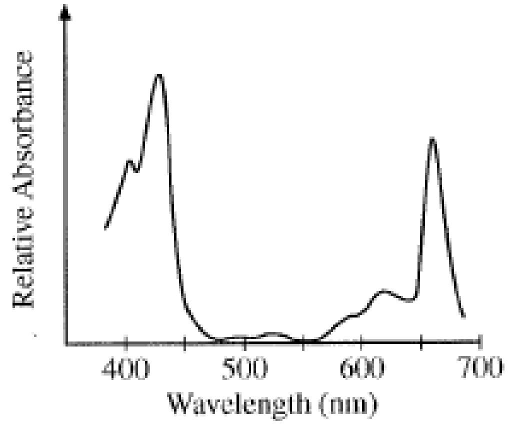
3. Using the pigment results & the introductory information presented, list the pigments in **ascending order of solubility** in the solvent used. Besides solubility, **what 2 other factors** might have caused the pigments to migrate at different rates?

4. For each pigment observed, write which colors & wavelengths (Textbook p.191) the pigments **absorb** and how this is related to their **color visible to us**. Explain the **evolutionary reasoning** for plants having **multiple pigments**.

FRQ Assessment Question



Graph I



Graph II

Color	Wavelength (nm)
Violet	380–450
Blue	450–475
Cyan	475–495
Green	495–570
Yellow	570–590
Orange	590–620
Red	620–750

An absorption spectrum indicates the relative amount of light absorbed across a range of wavelengths. The graphs above represent the absorption spectra of individual pigments isolated from two different organisms. One of the pigments is chlorophyll *a*, commonly found in green plants. The other pigment is bacteriorhodopsin, commonly found in purple photosynthetic bacteria. The table above shows the approximate ranges of wavelengths of different colors in the visible light spectrum.

(a) **Identify** the pigment (chlorophyll *a* or bacteriorhodopsin) used to generate the absorption spectrum in each of the graphs above. **Explain** and **justify** your answer.

(b) In an experiment, identical organisms containing the pigment from Graph II as the predominant light-capturing pigment are separated into three groups. The organisms in each group are illuminated with light of a single wavelength (650 nm for the first group, 550 nm for the second group, and 430 nm for the third group). The three light sources are of equal intensity, and all organisms are illuminated for equal lengths of time. **Predict** the relative rate of photosynthesis in each of the three groups. **Justify** your predictions.

(c) Bacteriorhodopsin has been found in aquatic organisms whose ancestors existed before the ancestors of plants evolved in the same environment. **Propose** a possible evolutionary history of plants that could have resulted in a predominant photosynthetic system that uses only some of the colors of the visible light spectrum.